Report
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An Evaluation Pattern for Antimacrofouling Procedures: *Limnoperna fortunei* Larvae Study in a Hydroelectric Power Plant in South America

The effects of global change and globalization of trade on the biosphere spur an increase in bioinvasions and their subsequent impact on ecosystems. Continental invading bivalves are important because of their impact on artificially-constructed structures. *Limnoperna fortunei* was first found in the Neotropical region in 1991. Since then it has dispersed upstream in the Plata and Guaíba basins at a rate of 240 km y^{-1}. This species causes macrofouling in a manner similar to that caused by *Dreissena polymorpha*. This paper describes the biology of *L. fortunei* larvae from a hydroelectric power plant in South America. We suggest the importance of knowing the biology of the invading species and the need to consider the settlement patterns and densities of larvae in each of the sectors of the facility in order to achieve a sustainable prevention/control of macrofouling. This study acquires a global significance under the assumption that *L. fortunei* will eventually invade North America and Europe.

INTRODUCTION

Limnoperna fortunei (Dunker, 1857) or golden mussel, is an invading bivalve belonging in the Mytilidae. It was recorded first in South America in 1991 (1). It entered the Río de la Plata estuary in ballast water carried overseas from Southeast Asia (2). This species shows characteristics similar to those of the marine members of the family, i.e., an epibyssate habit, which in turn originates a new microhabitat in the region (3), a unique reproductive biology (4–7), and a planktonic larvae measuring only a few microns (8, 9, for Asian populations; 10–12, for South American populations). All of these features are nontypical for native bivalves in the region and presumably enabled the wide dispersal of the golden mussel within South America (13, 14), the displacement of the native fauna, and the first case of freshwater macrofouling in raw water circuits of industrial plants recorded (in 1994) in South America (15). This mussel is presently the dominant species in hard substrates of the large rivers of South America, i.e., Paraná, Paraguay, Uruguay, and Guaíba (14–16), where it causes severe environmental impact (17). The presence of the golden mussel in freshwater in the region spurs great economic loss as it invades industrial areas of the *Mercado Económico del Sur* (MERCOSUR) (18).

This species shows morphofunctional characters similar to *Dreissena polymorpha* (Pallas, 1771), the zebra mussel. This similarity is so great, that both species have similar impact on aquatic ecosystems (19). Likewise, the invasive capability of the golden mussel is so high that in the past decades, after invading different Asian countries (e.g., Hong Kong and Japan), it began colonization of the Neotropical region at a rate of 240 km per year (20). These facts, together with a growing globalization of trade and the stress caused by global warming, suggest that *L. fortunei* will not only invade North America and Europe (16, 17, 21), but that this invasion may occur soon.

In 1998, *L. fortunei* was recorded for the first time in the Yacyretá hydroelectric power plant (YHPP) (15). This hydroelectric power plant is in an area with a subtropical climate appropriate for this bivalve; temperature, pH, and salinity, among other environmental parameters, are within range suitable for its ecological requirements (17). Since 1998, YHPP has mussel settlements in different sectors of the cooling circuit and, therefore, the system must be cleaned periodically. Between 1999 and 2001, experimental trials were carried out in YHPP with the aim of testing different antifouling treatments as alternatives to cleaning. For most of them, the environmental acceptability and the cost depend greatly on the biological features of the local population. Precise data on larval biology and settlement periods are needed to determine the most appropriate frequency to clean the walls or to choose the most suitable chemical treatment. The aim of this study was to describe the temporal biology of *L. fortunei* larvae in this artificially contructed environment.

MATERIAL AND METHODS

The present study was undertaken in YHPP. This is a binational hydroelectric power plant (Argentina-Paraguay), located on the upper Paraná river (27°29′S–56°44′W) (Fig. 1a). The average flow volume is 12 000 cubic meters per second. This plant has 20 turbines, and each turbine has a flow volume of 380 cubic meters per second. The reservoir lake covers an area of 1600 km^2 and is 342 km long.

Larvae of *L. fortunei* were quantitatively monitored every 2 weeks, between April 1999 and May 2001, with the exception of the samples taken between January and September 2000, which were taken monthly. Samples were collected by a plankton net (40 μm) sieving known volumes of water (30 L, the same volume used in other studies carried out in the subtropical region; e.g., 11) and concentrated to 250 mL. Representative aliquots were taken in the laboratory, larvae were counted, and the developmental stage identified under binocular microscope.

Two larval stages of *L. fortunei* were recognized: D larvae (corresponds to D larvae and preumboned veliger of 12) and umboned larvae (corresponds to an umboned veliger, pediveliger, and plantigrade of 12).

The samples were taken at different points (Fig. 1b):

i) three samples from the water intake structure (WIS) from turbines 1, 9, and 18; *ii)* six samples from different points of the cooling system (CS), including one before (CS1) and one after (CS2) the raw water auto-cleaner filter, one after the auto-cleaner filter of turbine 9 (CS3), one from the return water of turbine 9 (CS4), and one before (CS5) and one after (CS6) the sealed water system (from turbine 9); and *iii)* one sample from reservoir (R) (27°28′S–56°42′W).

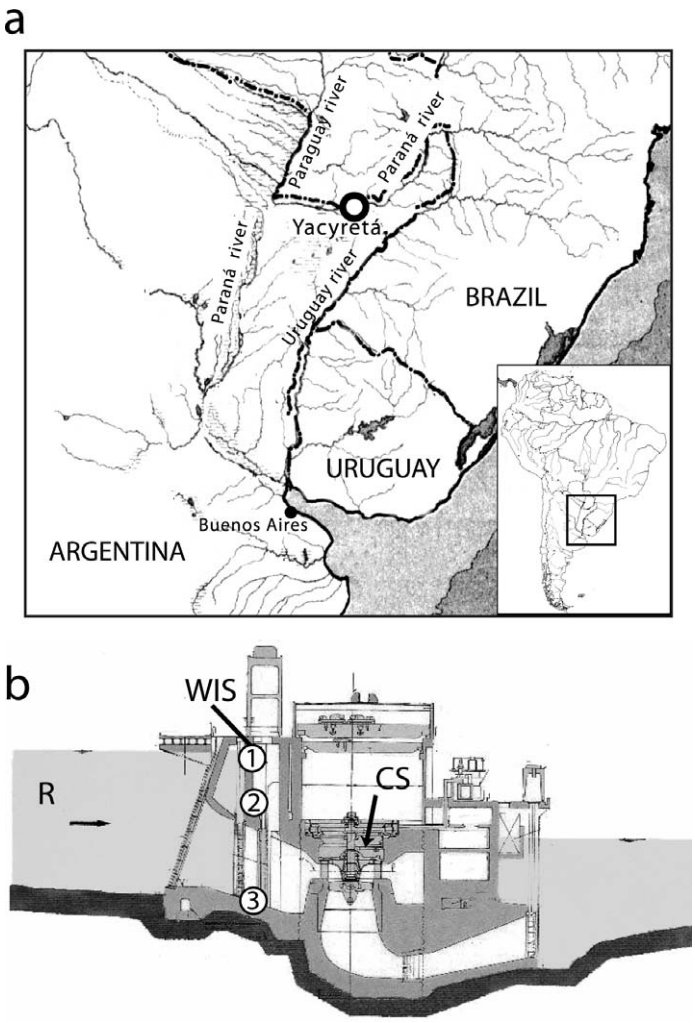

Figure 1. (a) Geographic location of the sampling area. (b) Sketch of the hydroelectric power plant studied; R: reservoir; WIS: water intake structure; CS: cooling system; *Limnoperna fortunei* adult densities, ① 170,400 ind m^{-2}, ② 248,200 ind m^{-2}, ③ 54,400 ind m^{-2}.

Larvae densities from different sites were compared using Kruskal-Wallis. Regression analysis was used for analysis between larva recruiting and density.

Larval settlement was studied between April and November 1999 and September 2000 to May 2001. Artificial substrates composed of fibrocement were used to quantify larval settlement. Two collectors (Fig. 2), each containing four rectangular plates (10 × 30 cm) were placed in the WIS 4 m below the surface. The collectors were lowered using chains. Every 2 weeks, the two collectors were removed and replaced with new ones. Shells measuring less than 5 mm in length were counted under a stereoscopic microscope.

Conductivity, total dissolved solids (TDS), pH, and temperature were measured at each sample point. Water velocity at the WIS of one of the turbines was measured using a Swoffer instruments 2100 microvane current meter.

RESULTS

Superficial water temperature recorded during this study varied between 15.3°C to 32.6°C in the reservoir (average value 24°C), between 16.3°C to31.8°C in the WIS (average value 23.8°C), and between 15.7°C to 31.9°C in the CS (average value 23.6) (Table 1, Fig. 3).

Water velocity on the walls of the WIS at 7 m depth varied between 0 m seg^{-1} and 0.03 m seg^{-1}, whereas at the center it ranged between 0.15 m seg^{-1} and 0.30 m seg^{-1}.

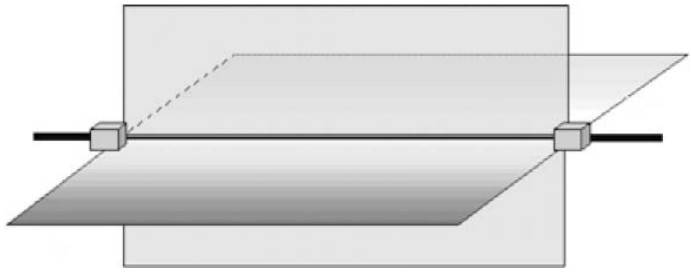

Figure 2. Samplers placed in the water intake structure used for recruitment studies.

D larvae and umboned larvae were present during the study most of the year, being absent only during 2–4 months. The presence of the larvae varied between the sample sites considered. Larvae from the CS were absent between July and August, both in 1999 and 2000; from the WIS between June and August, both in 1999 and 2000; and from the reservoir between June–September 1999 and June–August 2000 (Figures 3a–c).

The average density of larvae (total of D and umboned larvae) in the samples from the CS was 9.36 larvae L^{-1} (SD = 23.00; n = 252). The maximum value was 222.22 larvae L^{-1} observed on 28 December 1999, in the return water (CS4) from turbine 9. Larvae were not observed when water temperature was less than approximately 18–19°C (Fig. 3a).

The highest densities of larvae were found in the samples from the WIS (259.3 larvae L^{-1} on 26 November 1999 in the WIS of turbine 1). The average density in the WIS was 24.86 larvae L^{-1} (SD = 42.91, n = 126). Larvae were first observed in September, when water temperature was about 18°C; larval densities subsequently increased during the summer to reach the maximum values for the year. High densities were maintained between October 1999–March 2000 and December 2000–February 2001 (Fig. 3b). After this and before the water temperature began to drop, larval densities declined fast, i.e., during May 2000 and February 2001.

The mean larval density in the reservoir was the lowest observed (x = 7.79 larvae L^{-1} SD = 16.42; n = 42). The maximum values were recorded on 26 November 1999 and on 29 December 2000 (density = 74.07 larvae L^{-1}). D and umboned larvae were observed when water temperature was about 16–20°C (Fig. 3c). High densities were observed only during short periods between November and December when water temperatures began to increase toward maximum summer values. Thereafter densities declined before water temperatures began to drop.

A comparison of larval densities at different sampling sites is shown in Table 2. Larval density was significantly higher, according to the Kruskal-Wallis test, in the return water (CS4) than in the entrance of water to the cooling system (CS1). This difference is depicted in Figure 4a as comparative percentages, showing that along the CS, water becomes enriched in larvae. The WIS showed a higher larval density than the CS but the difference was not significant (Table 2, Fig. 4b). Larval density in the WIS was significantly higher than in the reservoir (R-WIS) (Table 2, Fig. 4c), suggesting that larvae entering the CS are not only those found in the reservoir, but that the WIS increases their number.

Regression between larval density and environmental parameters considered for each sampling site (Tables 1 and 3) were significant only for temperature ($P < 0.05$) in all sectors except the reservoir. The relation between the average larval density and settlement recorded in turbine 9 (Fig. 5) was significant ($R^2 = 0.206$; $P = 0.013$; $y = 262.66 + 0.45x$).

According to the Kruskall-Wallis test, the size of D larvae was significantly different between the reservoir and the WIS

Table 1. Average values (X), standard deviation (SD) and number of samples (n), for each of the environmental parameters considered at each of the sampling sites during the studied period.

	R			WIS			CS		
	X	SD	n	X	SD	n	X	SD	n
Temperature (°C)	24.01	4.91	38	23.84	4.21	117	23.62	4.19	234
Conductivity (μS cm^{-1})	44.08	3.86	36	47.58	3.75	117	47.16	3.23	234
Total dissolved solids (mg L^{-1})	23.26	2.53	36	23.74	1.95	117	23.48	1.79	234
pH	6.97	0.57	30	6.79	0.47	102	6.90	0.61	204

R = reservoir; WIS = water intake structure; CS = cooling system.

($H_{(3,\ N\ =\ 1147)} = 35.12$; $P < 0.001$): they were bigger. Whereas, the size of the umboned larvae was not significantly different ($H_{(3,\ N\ =\ 112)} = 2.40$; $P = 0.493$). The proportion of umboned/D larvae was bigger in the reservoir than in the WIS (WIS turbine 2 = 0.03; WIS turbine 9 = 0.12; WIS turbine 18 = 0.07; R = 0.22).

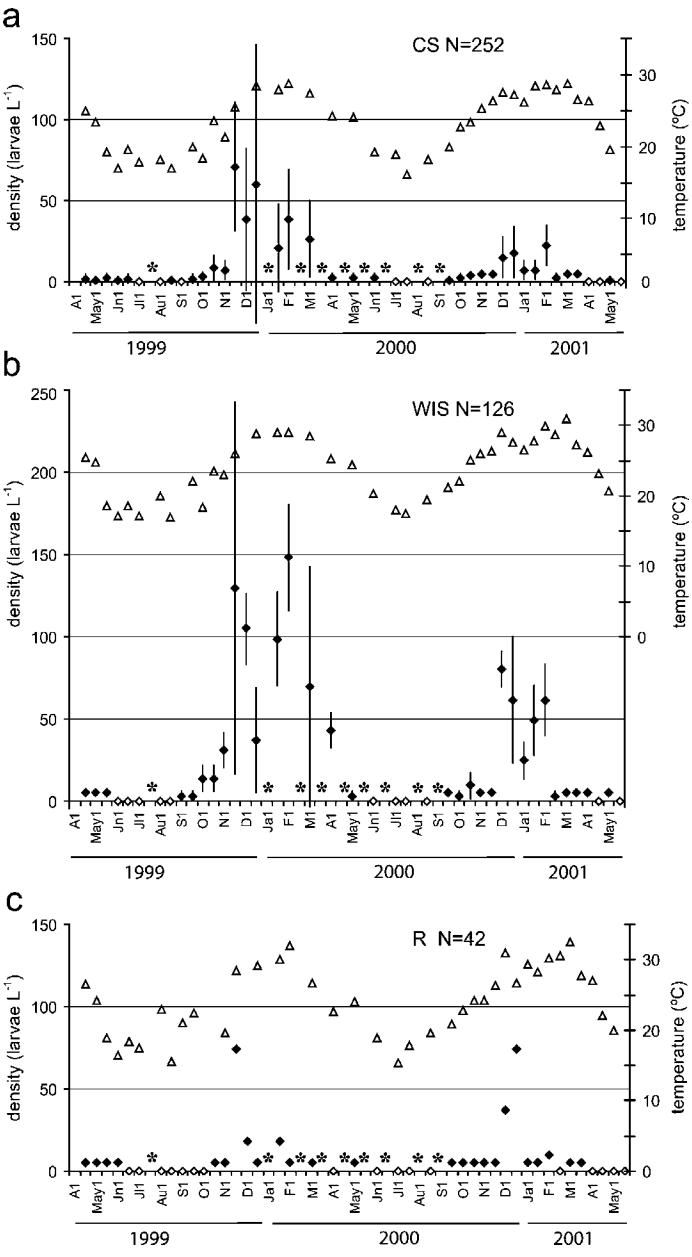

Figure 3. Temporal variation of larval density, standard deviation, and temperature in the different environments studied: (a) cooling system; (b) water intake structure; (c) reservoir. *sample missing; ♦ average density values; ◇ average density = 0; △ temperature.

DISCUSSION

During the 25 months that this study lasted, *L. fortunei* larvae were absent for 2–4 months each year depending on the sampling site considered and in coincidence with the lowest water temperate (15.3°C). The first larvae were recorded after the reproductive pause, when water temperature rose to 18–20°C (Fig. 3). Boltowskoy and Cataldo (22) and Cataldo and Boltowskoy (23) found that at two localities in Argentina (Central Atómica Atucha I on the lower Paraná river and in the Río de la Plata) that the thermal limit for the reproduction of this bivalve is approximately 16–17°C. These values are lower than those recorded in this study, in which water temperatures below 16–17°C were very rare throughout the year and therefore the populations were in reproductive pause during that time. This area lies in an area with subtropical climate.

Damborenea and Penchaszadeh (24) presented the results of a preliminary study of the reproductive cycle of *L. fortunei* in the reservoir of this hydroelectric power plant between November 1998 and October 1999. According to these results, spawning extended up to June/July. After this there is a period of gonad recovery in which the temperatures range from 20°C to 22°C. Ezcurra de Drago et al. (12) recorded larvae in the middle Paraná river and in the Salado del Norte river at temperatures above 18°C. However, it should be pointed out here that although water temperature appears to be the trigger of reproductive activity in golden mussels as it is with other aquatic invertebrates, by itself alone it is unable to induce spawning. Temperature is an important environmental feature that is accompanied by others, such as photoperiod, abundance of phytoplankton, etc., all of which together induce spawning (25, 26, among others).

Larval density was very variable at the different sampling sites in this study. Maximum values recorded were 259.3 larvae L^{-1} at one of the WIS sites in November 1999: 222.2 larvae L^{-1} in a sample within the cooling system on 28 December 1999 and 74.07 larvae L^{-1} in the reservoir in November 1999 and December 2000. These values surpass the maximum values

Table 2. A comparison of larval densities of *Limnoperna fortunei* between sample sites as Kruskal-Wallis value (H), degree of freedom (df), and probability (P).

Comparison	H	df	P
CS1-CS2	1.085	1, 82	0.298
CS5-CS6	0.050	1, 82	0.823
CS4-CS1	5.877	1, 82	0.015*
CS4-CS2	1.667	1, 82	0.197
CS4-CS3	0.439	1, 82	0.507
CS5-CS1	0.044	1, 164	0.834
R-CS	0.438	1, 82	0.508
WIS (turbine 1, 9, and 18)	0.148	2, 123	0.929
R-WIS	6.269	1, 164	0.012*
WIS-CS	2.980	1, 82	0.084

R = reservoir; WIS = water intake structure; CS = cooling system. *Significant values.

Table 3. Multiple regression (for each sector) among the larval density, temperature, pH, conductivity, and the total dissolved solids.

Sector	R^2	F	df	P
R	0.147	1.494	4. 26	0.239
CS5	0.221	2.884	4. 30	0.054
CS6	0.261	3.525	4. 30	0.027
CS3	0.437	7.750	4. 30	0.001
CS1	0.252	3.368	4. 30	0.031
CS2	0.547	12.080	4. 30	0.000
CS4	0.433	7.652	4. 30	0.001
WIS (Turbine 9)	0.486	9.475	4. 30	0.000
WIS (Turbine 18)	0.381	6.167	4. 30	0.002
WIS (Turbine 1)	0.370	5.886	4. 30	0.003

R = reservoir; WIS = water intake structure; CS = cooling system.

recorded by Cataldo and Boltowskoy (23), who mentioned densities of 26–28 larvas L^{-1} in Atucha and 33.7 larvae L^{-1} in the Río de la Plata. Despite these differences, seasonal peaks of maximum densities are approximately coincident, occurring between October and April.

Among the environmental parameters considered, larval density is significantly related only to water temperature, as observed by Cataldo and Boltovskoy (23). The highest larval density was found at the WIS. This would act structurally as a funnel that collects larvae from the surrounding areas in the

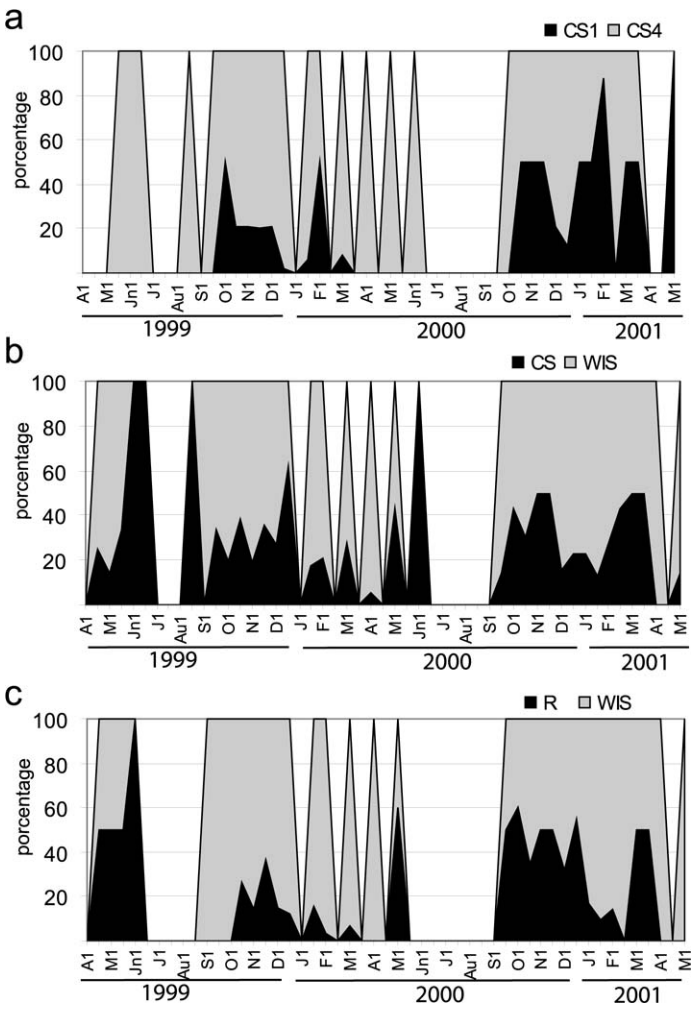

Figure 4. A comparison of larval densities (in percentages) at different sampling sites: (a) between the return system (CS4) and the entrance to the cooling system (CS1); (b) between the water intake structure (WIS) and the cooling system (CS); (c) between the water intake structure (WIS) and the reservoir (R).

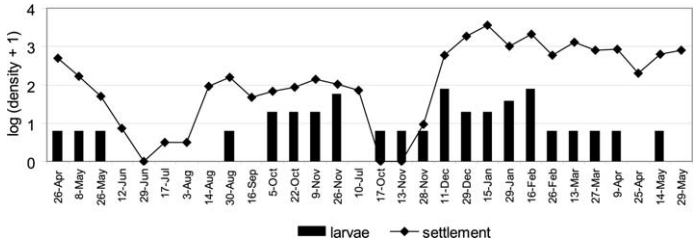

Figure 5. The temporal variation of larval average density (ind m^{-2}) and settlement (smaller than 0.5 mm length) recorded in turbine 9.

reservoir. Besides, it represents an important source of larvae, as its walls carry large numbers of golden mussel (14). These walls are concrete structures and the water velocity along them is nil, whereas a few centimeters away from the wall and at a depth of 7 m, the water velocity is only 0.03 m seg^{-1} and increasing toward the center of the structure. This enables a longer residence time of the larvae in the water near the walls of the WIS and facilitates the settlement. The densities of up 243 000 and m^{-2} at 10 m depth (17) (Fig. 1b), suggest that this environment acts as a "breeding chamber" (27) in which many of the larvae generated in this microhabitat never reach the high velocity areas of the system, moving only short distances within the WIS. The velocity of water contiguous to the WIS walls and along the walls of the CS pipes correspond to the abundant "dead-water sites" mentioned by Cataldo et al. (10) when referring to the cooling system of the facilities. On this basis, the residence time is long, giving the larvae present there the time necessary to settle among the byssus fibers of their congeners and the adequate conditions for their maturation and reproduction.

As observed in Figure 3a, the presence of larvae in CS was similar to that recorded from the reservoir and WIS. Larval densities in this pipe system are significantly higher (according to the Kruskal-Wallis test) once the water has completed its circuit through the cooling system (return water or CS4) than at the beginning of the pipe system (before the autocleaning filter or CS1) (Fig. 4a) This result suggests the existence of stable and reproductively active populations that shed larvae into CS. Likewise, the environmental conditions within the water system are better when compared with the natural environment outside. Therefore, transformation of D larvae into umboned larvae occurs faster (e.g., the D larvae are smaller in size than those found in the reservoir) and therefore more frequent in this environment.

With the high densities of adults of *L. fortunei* in the WIS, the larval densities, the environmental stable conditions, and available substrate in this sector, the umboned larvae settle quickly. In the reservoir, where the environmental conditions are less stable and the available substrate lesser, the umboned larvae need more time to settle, so their proportion in relation to the D larvae is greater.

Limnoperna fortunei is presently a severe problem for industrial plants using water from the Plata and Guaíba basins in South America (15), because they cause macrofouling and thus important economic losses (28). The environmental acceptability and the cost of controlling this bivalve depend greatly on the biological features of the local populations of *L. fortunei*. Therefore, data on larval biology and settlement periods are necessary for the design of control treatment strategies in the invaded facilities. Adaptive and reproductive variations in this invading species are important as they allow survival when colonizing natural environments with diverse characteristics, but also when invading artificial environments. For instance, it is known that the spatial range of the larvae is heterogeneous, whether in lentic or lotic environments (12) and

that *L. fortunei* shows pronounced geographic variations in its reproductive cycle (24, 29). In this contribution we contrast features such as the changes in the thermal limits necessary for reproduction of the species in different environments with temperate/subtropical climates, as well as the greater densities of larvae produced by the golden mussel as it ranges into a subtropical climate. These data, together with other pertaining to the biology of *L. fortunei* are necessary for a sound choice of antifouling treatment that is efficient from economic and environmental perspectives. They are also necessary to establish the most appropriate frequency for cleaning the walls of the water intake systems and their piping systems. The results obtained point toward the importance of sectorization of the facilities. Population dynamics are different in each sector and therefore these become microhabitats in which the antifouling treatments should be specific in order to achieve a sustainable strategy for the entire system.

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